Midlife gene expressions identify modulators of aging through dietary interventions

Bing Zhou\textsuperscript{a,b,c,1}, Liu Yang\textsuperscript{d,1}, Shoufeng Li\textsuperscript{i}, Jialiang Huang\textsuperscript{a,b,c}, Haiyang Chen\textsuperscript{n}, Lei Hou\textsuperscript{a,b,c}, Jinbo Wang\textsuperscript{a,e}, Christopher D. Green\textsuperscript{a}, Zhen Yan\textsuperscript{n}, Xin Huang\textsuperscript{a}, Matt Kaeberlein\textsuperscript{b}, Li Zhu\textsuperscript{n}, Huasheng Xiao\textsuperscript{a}, Yong Liu\textsuperscript{d,2}, and Jing-Dong J. Han\textsuperscript{a,2}

*Chinese Academy of Sciences Key Laboratory for Computational Biology, Chinese Academy of Sciences—Max Planck Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; \textsuperscript{a}Centre for Molecular Systems Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; \textsuperscript{b}Graduate University, Chinese Academy of Sciences, Beijing 100049, China; \textsuperscript{c}Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China; \textsuperscript{d}State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; \textsuperscript{e}Departments of Medicine—Cardiovascular Medicine and Pharmacology and Center for Skeletal Muscle Research, Robert M. Berne Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908; \textsuperscript{f}Department of Pathology, University of Washington, Seattle, WA 98195; and \textsuperscript{g}Key Laboratory of Systems Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

AUTHOR SUMMARY

Dietary interventions are effective ways of extending or shortening lifespan. By examining midlife hepatic gene expressions in mice under different dietary conditions and their correlation with lifespans, we not only identified well-known diet-responsive, lifespan-regulating pathways, such as the mitochondrial genes, but also predicted some unique or underappreciated ones, such as the peroxisomal biogenesis pathway. We found that lowering the expression of peroxisome proliferation genes decreased cellular peroxide levels and extended the lifespan of fruit flies and worms. These findings show that transcriptional changes resulting from dietary interventions can effectively reflect causal factors in aging and identify longevity pathways.

Aging-related gene expressions have been examined for various organisms, and many genes and biological functions that change with age have been revealed. Moreover, genetic, dietary, or reproductive interventions have been shown to effectively modulate lifespan and aging (1, 2). Caloric restriction (CR) is the best-studied of these interventions and is reported to prolong both mean and maximal lifespans in most organisms examined (1, 2). In contrast, high-fat/high-calorie diets cause health problems and shortened lifespans in mice (3, 4). Exercise, however, can prevent some age-related declines (5). It is, therefore, not surprising that nutrient and energy-sensing pathways have been identified as key regulators of lifespan and aging.

To further define the mechanisms by which these interventions modulate aging, we subjected mice to six different diet/energy regimens (30 mice per group) that led to the following order of increasing lifespan: high-fat (HF) diet fed ad libitum, HF diet combined with voluntary exercise (HF + Ex), low-fat (LF) diet fed ad libitum, LF diet with voluntary exercise (LF + Ex), HF diet combined with 70% CR (HF + CR), and LF diet with 70% CR (LF + CR). In addition to lifespan, health span parameters were assessed for each cohort, including liver and metabolic functions. Gene expression profiles were obtained at midlife before the increase in mortality.

In this context, we first asked if the six different dietary groups give rise to different lifespans according to their energy input and output levels. We also asked whether we could predict the lifespan differences across these groups from the midlife liver phenotype and hepatic gene expressions and finally, whether the genes or pathways that predict the lifespan differences are regulators of lifespan (Fig. P1). The fact that all of the intervention experiments were carried out in parallel rather than in different laboratories with variable or noncomparable conditions enabled us to conduct an integrative analysis free of system variations in the data. We were, thus, able to search for common target genes of different dietary interventions that contribute to the consequent lifespan differences through changes in their gene expression levels (Fig. P1). We found that dietary interventions led to concordant changes in aging-related physical and physiological phenotypes. These changes were reflected by midlife gene expression differences corresponding to six different dietary regimens.

Our results indicate that midlife liver gene expressions showing positive or negative correlation with mean lifespan across the six groups indeed constituted many genes previously implicated in aging. Importantly, the overall correlation of incremental changes in hepatic gene expression of a molecular pathway to the mean lifespan changes under these conditions could be used to identify lifespan-modifying or -regulating genes. We verified this

---

**Fig. P1.** Six cohorts of mice with different energy input and output levels were designed to enable an integrative analysis free of system variations in the data. With this methodology, we investigated whether dietary interventions under the six different conditions target the same set of genes, with expression changes that may modify lifespan. Male mice at 5 wk of age (n = 30/group) maintained on an LF or HF diet were fed ad libitum, subjected to CR (LF + CR or HF + CR; fed daily 70% of the food consumed by the ad libitum group), or permitted voluntary wheel-running exercise (LF + Ex or HF + Ex). At the midlife age of 62 wk, eight mice from each group were killed for analyses of blood and tissues and hepatic gene expression profiling by microarray. The remaining 22 animals in each group were used for lifespan determination.

---


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession nos. GSE36836 and GSE36838).

1B.Z. and L.Y. contributed equally to this work.

2To whom correspondence may be addressed. E-mail: luy@sibs.ac.cn or jdhan@genetics.ac.cn.


Cite this Author Summary as: PNAS 10.1073/pnas.1119304109.
conclusion in two ways. First, we showed that known longevity-modifying genes were enriched among genes and pathways with expression that was correlated or anticorrelated with lifespan. Second, we predicted that high expression of peroxisomal biogenesis genes might negatively influence lifespan and verified this prediction experimentally in Drosophila melanogaster and Caenorhabditis elegans, two different model organisms commonly used in aging studies. These results implicate peroxisomal biogenesis as a key determinant of longevity.

Our approach of directly measuring lifespan along with measuring gene expressions under different dietary and other metabolic conditions permitted the identification of pathways that may directly explain lifespan changes instead of other phenotypic changes. Furthermore, our dietary perturbation-based method of gene expression analysis has successfully identified peroxisomal biogenesis factors as an important class of conserved, longevity-modifying genes. This finding provides insight into the molecular mechanisms linking diet, disease, and aging.